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Surface-Assisted Self-Assembly Strategies Leading to Supramolecular Hydrogels

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Localized molecular self-assembly processes leading to the growth of nanostructures exclusively from the surface of a material is one of the great challenges in surface chemistry. In the last decade, several works have been reported on the ability of modified or unmodified surfaces to manage the self-assembly of low-molecular-weight hydrogelators (LMWH) resulting in localized supramolecular hydrogel coatings mainly based on nanofiber architectures. This Minireview highlights all strategies that have emerged recently to initiate and localize LMWH supramolecular hydrogel formation, their related fundamental issues and applications.

1. Introduction

Since the middle of the last century, functionalization of surfaces has emerged as a convenient method for controlling interactions between a material and its surrounding environment.[1] Many effective approaches were developed covering a large spectrum of applications. Currently, the recent boom of nanoarchitectonic systems paves the way for the design of chemical systems located on surfaces and responsive to several stimuli from the surrounding medium.[2] This emerging field is already considered as a hot topic in chemistry because it allows providing original smart functionalities to materials for various applications. The central question in this field is how to 1) direct and control a nanostructure buildup that is 2) initiated exclusively from or on a surface. Point (1) is a long standing problem in surface science. It is, for example, related to crystal growth in living organisms, which is initiated through a templating effect on surfaces.[3,4] With the almost simultaneous development of scanning tunneling microscopy[5] and supramolecular chemistry,[6] the ordering of organic molecules on surfaces into 2D crystals has also become a hot topic.[7] Such ordering is induced through the subtle interplay between lateral interactions and molecule/surface interactions.[8,9] It is addressed through various interactions including coordination bonds,[10] hydrogen bonds, or van der Waals forces,[7] with interesting applications in catalysis and[11] chiral recognition,[12] for example. When extending towards the 3rd dimension, great effort has been made to develop organized molecular and macromolecular films extending from the nanometer to the micrometer range. Langmuir–Blodgett,[13] self-assembled monolayers,[14] and polyelectrolyte multilayers[15] are only a few examples along this line. With the development of supramolecular hydrogels in the bulk, a new issue arose that also addresses point (2): how to initiate supramolecular hydrogels exclusively near a surface? The answer to this question is central in biology in which many processes, such as actin filaments on focal points during cell adhesion[16] and microtubule spindle formation on centrosomes for chromosome separation,[17] involve spatio-temporal control over directed self-assembly. This Minireview will cover the advances in this new topic. We will restrict ourselves to low-molecular-weight hydrogelators (LMWH), which constitute by themselves an entire field in supramolecular chemistry and present multiple similarities, as far as their interactions are concerned, with the building blocks of the previously mentioned biological networks.

LMWHs self-assemble in solution through noncovalent bonds and lead to the formation of fibers.[18] Above a critical LMWH concentration (critical gelation concentration, CGC) these fibers form a supramolecular hydrogel.[19] Localizing the formation of such a gel exclusively near a surface is challenging and two main routes were followed to address this issue: 1) One can deposit or favor the deposition of LMWH molecules on the surface. The surface then plays the

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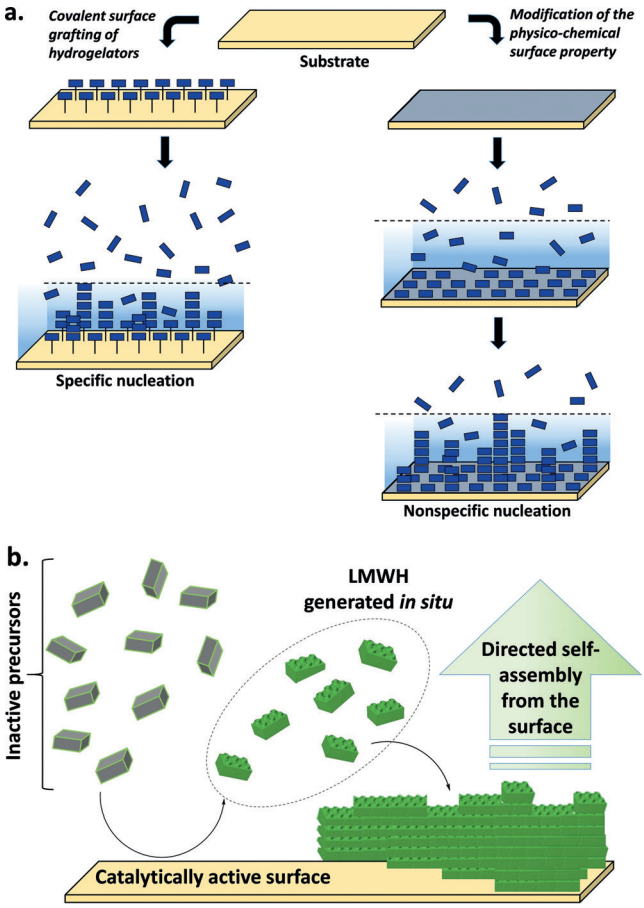


Figure 1. a) Schematic of surface modifications that lead to the localized self-assembly of LMWH in the close vicinity of the surface. b) Catalytically active surface transforms inactive precursors in solution into efficient LMWHs. The local accumulation of the latter in the close vicinity of the surface induces the directed self-assembly.

role of "seed layer" that can initiate the local formation of a confined supramolecular hydrogel at LMWH bulk concentrations far below the CGC (Figure 1a). 2) One can also produce the hydrogelator molecules directly at or near the surface so that the CGC is reached exclusively near the surface (Figure 1b). Both approaches were followed in different ways and will now be reviewed.

2. Localized Supramolecular Hydrogelation of LMWH Induced by a Seed Layer

The surface-induced localized gelation of LMWH at concentrations far below the CGC was first observed by B. Xu in 2002 on cells[20] and demonstrated by J. C. Tiller in 2003.[21,22] The chemical system used by Tiller was based on an anionic hydrogelator in the presence of a cationic surface. The hydrogelator was an aromatic amphiphile dye (1-(2-*n*-hexylphenylazo)-2-hydroxy-6-naphtalenesulfonate = OHD) and the substrate was a glass modified with aminopropyltriethoxysilane (Figure 2a). At acidic pH, the surface was positively charged because of the presence of ammonium groups ($-NH_3^+$) while OHD was negatively charged regardless of pH because of the sulfonate group ($pK_a < 0$). When the so-

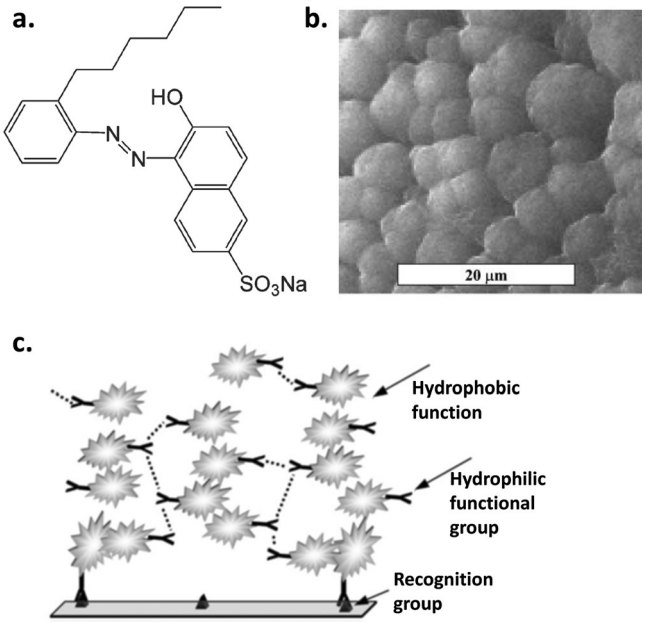
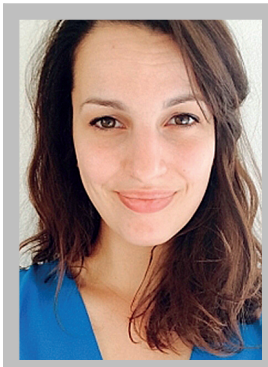


Figure 2. a) Chemical structure of the aromatic amphiphilic dye (OHD). b) Broccoli-like structure of the OHD hydrogel formed on a modified-glass surface observed by environmental scanning electron microscopy (ESEM). c) Model proposed to explain the localized gelation on a surface through electrostatic attraction of hydrogelators onto the surface. Reproduced from Ref. [22] with permission of The Royal Society of Chemistry.



Cécile Vigier-Carrière joined the Charles Sadron Institute in 2013 and received her Ph.D. in 2016 at the University of Strasbourg. Her Ph.D. research was on the control of spatiotemporal self-assembly nucleation of peptides. Her current research interest includes self-assembly and gelation for biological applications and the development of soft-structured catalyst support for industrial applications.

Fouzia Boulmedais received her Ph.D. in physical chemistry from University Louis Pasteur in Strasbourg (France) in 2003. Her postdoctoral research at ETH-Z in Zürich (Switzerland) and MPI in Golm (Germany) focused on the electrochemical response of multilayers of polyelectrolytes. She joined the Institut Charles Sadron in Strasbourg as a CNRS researcher in 2006. Her current research involves polyelectrolyte films, including their application in biomaterials and tissue engineering and their buildup by an electrical stimulus.

modified glass substrate was brought into contact with a highly diluted OHD solution, in fact 50-fold below the CGC, the dye was capable of gelling exclusively on the positively charged surface, forming a broccoli-like structure (Figure 2b). This was explained by a local increase of the concentration of gelator molecules because of electrostatic interactions (Figure 2c).

A second way to induce localized formation of a LMWH hydrogel is to adjust the hydrophilicity/hydrophobicity balance or the charge of the surface in order to favor nonspecific adsorption of the hydrogelator molecules onto the surface through weak interactions. The high concentration of adsorbed hydrogelator molecules then favors self-assembly and gel formation at the substrate. Along this line, the initiation of the self-assembly of C_{14}-cytidine (Figure 3a) was studied on two kinds of glass surfaces exhibiting either hydrophilic groups, that is, hydroxy groups (OH-surface) or hydrophobic phenyl moieties (Ph-surface)[23] (Figure 3b). For both samples, the surface-assisted self-assembly of C_{14}-cytidine formed supramolecular fibrous networks. However, analysis of the hydrogels by atomic force microscopy (AFM) revealed different physical and mechanical properties (Figure 3c). Gels self-assembled on Ph-surfaces presented fibers of larger diameters and were stiffer than fibers of gels assembled on OH-surfaces.

Another way to increase the propensity of LMWH to self-assemble into hydrogels locally is by grafting hydrogelators directly onto the surface (Figure 1a, left). This strategy was introduced in 2011 by using an aromatic peptide amphiphile (PA) as hydrogelator.[24] The peptide Fmoc-FF-OH (Fmoc = fluorenylmethyloxycarbonyl, F = phenylalanine) was grafted covalently onto a silica wafer surface (Figure 4). Immersion of the seed surface into an aqueous solution of Fmoc-FF-OH resulted in the formation of nanorods displaying antiparallel β-sheet structures. Increasing the concentration of Fmoc-FF-OH in solution generated a higher density of nanorods growing from the surface. Thus, hydrophobic interactions and π–π interactions drive the recognition between Fmoc-FF-OH building blocks and Fmoc-FF-OH sequences immobilized on the surface and control the local self-assembly of peptides. The formation of nanorod structures observed exclusively on the surface was explained by the immobilized Fmoc-FF-OH moieties acting as nucleation sites.

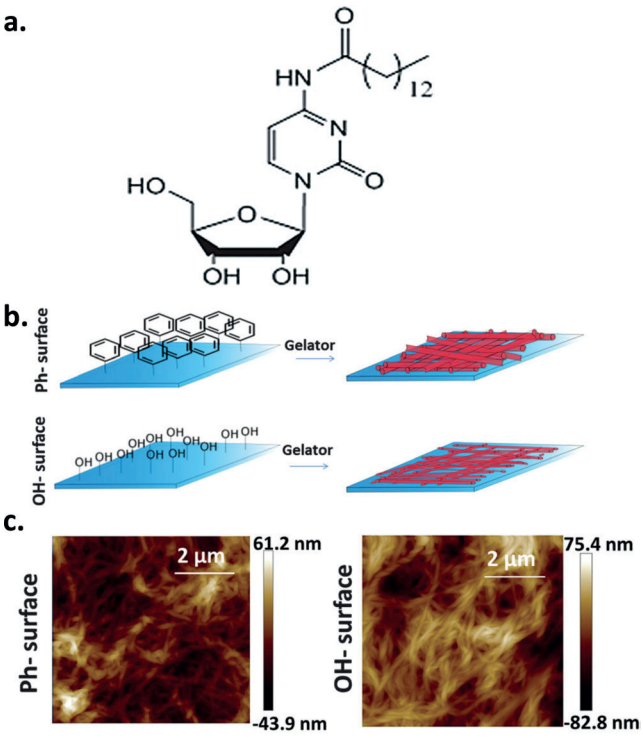


Figure 3. a) Chemical structure of the hydrogelator C_{14}-cytidine. b) Schematic of the two glass surfaces studied exhibiting either hydrophobic or hydrophilic properties. c) When both substrates are brought in contact with a C_{14}-cytidine solution, a distinct fibrillar network nucleates and grows in each case. Reproduced from Ref. [23] published by The Royal Society of Chemistry.

3. Surface-Induced Self-Assembly through the Localized Production of Hydrogelators

3.1. Hydrogelator Production through Local Decrease of pH

A second strategy to control the self-assembly of hydrogelators exclusively at an interface is based on generating the LMWH directly at or near the surface. Generating hydrogelators near the surface can be achieved by generating, at the surface, chemical species that diffuse from the surface towards the bulk and that switch molecules present in solution from a non-self-assembling state into a self-assembling one (Figure 1b). In the case of LMWH, the reported

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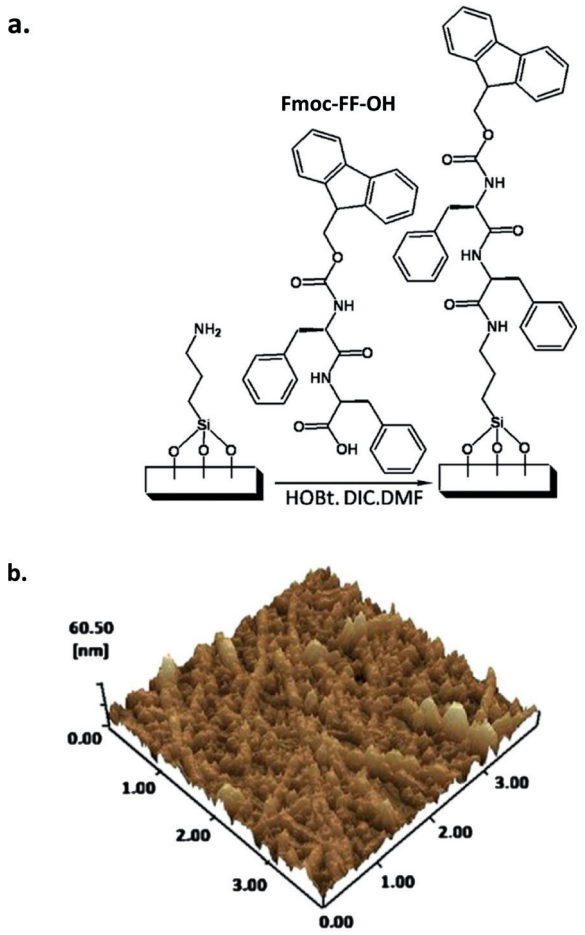

Figure 4. a) Chemical modification of a silica wafer through silanization with aminopropyltriethoxysilane, followed by the coupling with Fmoc-FF-OH. b) Three-dimensional AFM image (4 μm × 4 μm) of the resulting supramolecular hydrogel. Reprinted from Ref. [24], Copyright 2011, with permission from Elsevier.

systems used protons and LMWH presenting weak acid groups. An acidic flow directed from the surface then protonates the LMWH and thus changes its hydrophilicity/hydrophobicity balance, inducing self-assembly.

In 2010, Cameron developed a convenient method to direct the self-assembly of an aromatic peptide amphiphile (PA), Fmoc-LG-OH (L = leucine, G = glycine), into an ultrathin hydrogel membrane on an electrode (Figure 5a).[25] The self-assembly of the dipeptide hydrogelator was triggered in response to a decrease of pH generated at the electrode through the electrochemical oxidation of hydroquinone into 1,4-benzoquinone, releasing two protons per oxidized hydroquinone (Figure 5b). The adjustment of the current intensity affects the pH near the surface and thus impacts the Fmoc-LG-OH self-assembly, allowing for a precise control of the growth of a fibrous network (Figure 5c,d).

In another contribution, the same authors first produced the protons electrochemically in the presence of the peptides until a gel of 80–100 nm thickness was reached. They then stopped the electrochemical stimulus still in the presence of the Fmoc peptides and observed that the gel continues to grow slowly at least over 48 h.[26] It was suggested that the

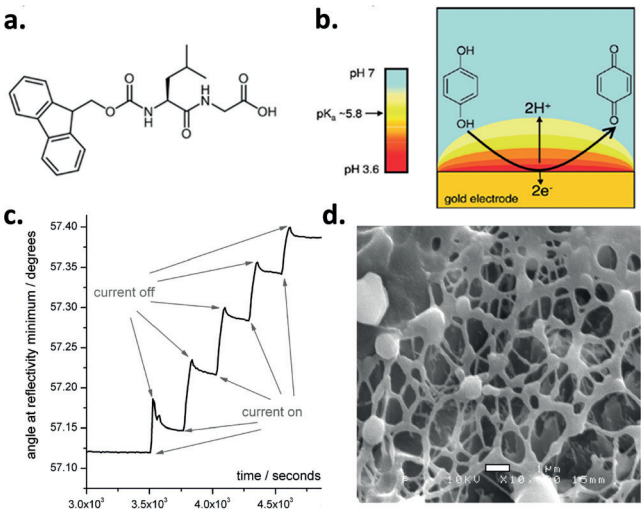

Figure 5. a) Chemical structure of the aromatic PA, Fmoc-LG-OH. b) Electrochemical oxidation of 1,4-hydroquinone to generate a gradient of protons going from the bottom to the top. c) SPR experiment showing the stepwise growth of the dipeptide Fmoc-LG-OH hydrogel with each 60 s current pulse. d) Cryo-SEM image of the top surface of an electrochemically grown gel film. Scale bar = 1 μm. Reprinted with permission from Ref. [25]. Copyright 2010 American Chemical Society.

initial electro-stimulated gel on the surface played the role of a seed layer for the continuation of the gelation process observed without continuous stimulus (Figure 6a). This was demonstrated by bringing a neutral solution of non-assembling peptides Fmoc-LG-OH into contact with the initial hydrogel, resulting in the formation of a supramolecular hydrogel, of millimeter thickness, constituted of large and long fibers (Figure 6b).

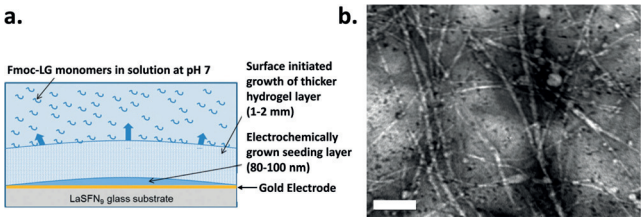

Figure 6. a) Schematic representation of the surface-initiated growth of a Fmoc-LG-OH based film. b) TEM image of the seed layer grown electrochemically onto the TEM grid (scale bar = 20 nm). Reproduced from Ref. [26] with permission of The Royal Society of Chemistry.

To explain this second gel buildup, it was suggested that residual protons trapped in the initial gel lowered the pH at the interface of the gel and shifted the pK_a of Fmoc-LG-OH, which self-assembled.[26] It must be noted that horse radish peroxidase (HRP) could also be successfully embedded into the second hydrogel layer, affording a catalytic function to the hydrogel. This result highlights the possibility offered by this strategy for the design of biosensors.

This electrochemical pH decrease generated from a surface also allowed the spatially resolved self-assembly of carbazole-protected amino-acids.[27] Alanine *N*-protected

with carbazole groups (Carb-Ala) led to fibrous structures that could be electropolymerized in a second step; the internal self-assembled architecture of the hydrogel played the role of template. The resulting conjugated electrochromic polymers presented a different structure from that obtained by direct electropolymerization without the templating gel phase.

The self-assembly of another hydrogelator initiated locally by the electrochemical oxidation of hydroquinone was recently investigated using patterned electrodes.[28] By using the *N*-protected phenylalanine with Fmoc group, Fmoc-F-OH (Figure 7a), Payne extended the electrochemical control over LMWH self-assembly by studying the reversibility of the assembly/disassembly process of hydrogelators. Similarly to Cameron, the self-assembly of Fmoc-F-OH was locally initiated through the electrochemical production of protons.[25–27]

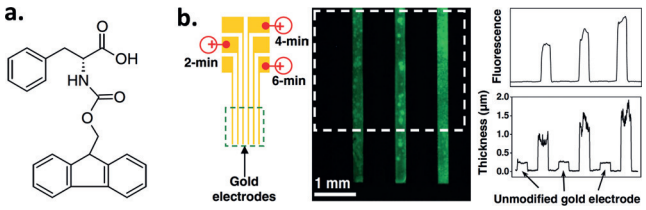

Figure 7. a) Chemical structure of the Fmoc-F-OH. b) Spatial-selectivity of Fmoc-F-OH electrodeposition. A chip with patterned gold electrodes (250 μm wide gold lines patterned onto a silicon wafer) demonstrates spatial selectivity of electrodeposition of 10 mM Fmoc-F-OH (0.005 M fluorescein-labeled dextran was included for visualization). Reproduced with permission.[28] Copyright 2011, Wiley.

The spatially directed self-assembly of Fmoc-F-OH was implemented by using a gold-electrode made of independent indentations. Each electrode indentation was partially immersed in a solution of Fmoc-F-OH with fluorescein-labeled dextran (fluorescent probe) and subjected to an anodic current. The protected amino acid, Fmoc-F-OH, self-assembled into nanofibers constituting the gel backbone. Selective control of the self-assembly in space (normal and lateral direction) and time was obtained by the timely localized application of the electrical signals (Figure 7b). A reversible assembly/disassembly of the hydrogel was obtained on an electrode chip. The gel disassembled in response to a cathodic current that increased the pH in the proximity of the electrode surface. When simultaneous cathodic and anodic currents were addressed to adjacent electrodes, spatially-specific assembly and disassembly of Fmoc-F-OH were observed on the same chip. This spatial and temporal control over the proton gradient is the key process to pattern reversible assembly and disassembly of aromatic *N*-protected amino acids.

In 2014, Adams and Cameron proposed to electrochemically produce protons with hydroquinone to trigger spatially and temporally resolved multi-component gels.[29] By using different aromatic peptide amphiphiles present simultaneously in solution, the chemical constitution of a gel could be controlled. By changing the electrochemical parameters controlling the oxidation of hydroquinone, they tuned the production of protons and thus the self-assembly propensity of peptides presenting different pK_a values to self-assemble. Aromatic dipeptides **1** and **2** with $pK_{a_1} = 6.6$ and $pK_{a_2} = 5.0$, were selected as building blocks to design multi-component hydrogels (Figure 8a). Fine adjustment of the pH near the electrode surface permitted reaching pK_{a_1} in the first place. LMWH **1** self-assembled to form a 3D network of fibers entrapping both water and the second non-self-assembling LMWH **2** (Figure 8b). Then, a continuous decrease of pH initiated the self-assembly of LMWH **2** to finally form an interpenetrated multi-component hydrogel. The chemical composition of the gel could be controlled temporally and spatially through the fine adjustment of electrical inputs.

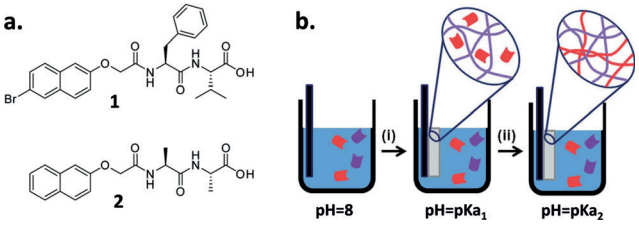

Figure 8. a) Chemical structures of dipeptide-based LMWH **1** and **2**. b) Schematic showing the sequential assembly of two LMWH **1** and **2** in a multicomponent system. Reproduced from Ref. [29] with permission of The Royal Society of Chemistry.

3.2. Surface-Confined Acidic Catalysis to Generate the LMWH In Situ

In 2014, van Esch[30] developed an original approach in which confinement of acidic catalysts (sulfonic acid groups) on the surface decreases the pH locally, which catalyzes the triple condensation of trihydrazine **3** with three aldehyde compounds **4** to afford a trihydrazone-based hydrogelator **5** (Figure 9a). This resulting LMWH is not stable in solution because of the reversible feature of the hydrazone bond. However, if the concentration of **5** reaches a critical threshold near the surface, hydrogelators self-assemble spontaneously into fibers through π–π stacking and hydrogen bond interactions to form a localized gel (Figure 9b). Thus, the catalytic rate of hydrazone bond formation is the key parameter to control the rate of the self-assembly and also to modulate the morphology and mechanical properties of the fibrous network.

3.3. Surface-Confined Enzyme Catalysis to Generate the LMWH In Situ

Since 2004, enzyme catalysis emerged as a valuable trigger to spontaneously and selectively transform non-assembling precursors (inactive) into efficient LMWH in the bulk.[31] Despite the fact that enzyme-assisted self-assembly is widespread in nature to achieve spatial control over complex architectures, the immobilization of enzymes onto a surface to produce LMWH locally and achieve surface-localized hydrogel growth is a field that emerged very recently (Figure 1b).

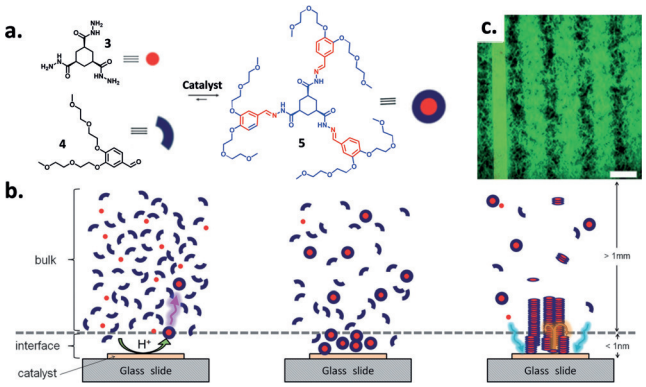

Figure 9. a) Catalytic formation of trihydrazone-based hydrogelator **5** from the soluble building blocks **3** and **4** leading to growth of nanofibers, which trap the surrounding water to form a supramolecular hydrogel. b) Schematic of **5** self-assembly oriented by the catalytic activity of the surface. c) Confocal microscopy image of patterns, composed of self-assembled fibers of **5**, which reflect the stamped catalytic pattern. White line, width = 10 μm and spacing = 15 μm (scale bar = 20 μm). Reproduced with permission.[30] Copyright 2014, Wiley.

Enzymes used as catalysts ensure a permanent catalytic active surface that, in the presence of inactive precursors, leads to a continuous generation of self-assembling molecules (LMWH). This autonomous control over the rate of LMWH formation confined to the surface contributes to the development of smart surfaces that are able to direct the organization of matter in space and time.

In 2009, Ulijn was the first to immobilize an enzyme, thermolysin, covalently linked on a surface in order to induce local self-assembly of a mixture composed of Fmoc-L-OH and the diamino acids LL-OH (L_2) (Figure 10a).[32] Thermolysin is able to both catalyze the coupling reaction between Fmoc-L-OH and diamino acids L_2 and hydrolyze amide bonds, leading to a statistical distribution of oligopeptides Fmoc-L_n-OH. These resulting aromatic peptide amphiphiles self-assemble to form fibers.

During the early stages of the self-assembly process nanofibers starting from the expected aggregates of enzymes were observed (Figure 10). Confining enzymes onto a surface can thus be a strategy to locally nucleate and grow LMWH-based fibers at an interface. This strategy, based on thermolysin and Fmoc-L-OH/dipeptide L_2, was also used by Williams et al. in 2011 to entrap a protein, laminin, inside a localized peptide-based hydrogel,[33] which can be of interest to treat genetic diseases involving the laminin gene family.

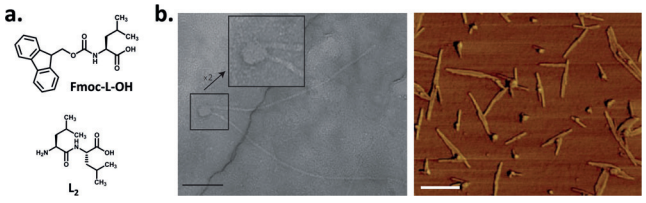

Figure 10. a) Chemical structures of Fmoc-L-OH and L_2. b) TEM image observed with fiber propagation, demonstrating confined fiber growth from spherical structures (left, scale bar = 100 nm) and the same process visualized by AFM phase imaging (right, scale bar = 250 nm). Reprinted by permission from Springer,[32] Copyright 2009.

Recently, Ulijn showed the effect of the anchoring strength of an enzyme on a surface on the self-assembly process.[34] Using again thermolysin and the system Fmoc-T-OH/F-NH_2 (T = threonine, F-NH_2 = phenylalanine with a carboxamide group at C-terminal position), forming the hydrogelator Fmoc-TF-NH_2 in situ, they showed that when the enzyme is not covalently fixed on the substrate and if the rinsing is not strong enough to remove the weakly adsorbed enzymes, the self-assembly leads to gelation in the bulk but in the proximity of the surface because the enzyme desorbs from the surface.

Localized enzyme-assisted self-assembly leading to a localized gel formation requires that the concentration of LMWH produced from the surface by the immobilized enzymes reaches a critical concentration at which localized gelation starts. When a too-low initial concentration of precursors is present near the active surface, no gel forms. Using alkaline phosphatase (AP) adsorbed on a surface and the Fmoc-FFY(PO_4^{2-})-OH peptide (Y = tyrosine), Vigier-Carrière et al. circumvented this difficulty by modifying the surface with a thin nanoarchitectured film including AP and a layer of polyelectrolytes bearing LMWH (seed layer) (Figures 11a,b).[35] AP dephosphorylates Fmoc-FFY(PO_4^{2-}) into Fmoc-FFY-OH, which self-assembles at concentrations lower than the CGC for gelation in the bulk, leading to a hydrogel underpinned by a nanofiber network that evolved over time (Figure 11). Varying the density of enzymes in the nanofilm or the surface density of the seeding peptides on the surface provided different kinds of nanofiber architectures (Figure 11c).

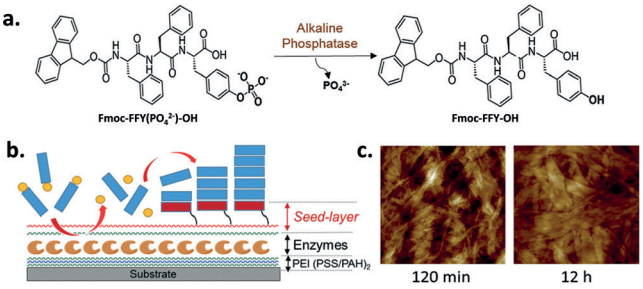

Figure 11. a) Chemical structure of Fmoc-FFY(PO_4^{2-})-OH and the resulting LMWH Fmoc-FFY-OH produced with AP. b) Schematic of the nanoarchitectured film on a surface, allowing the localized enzyme-assisted self-assembly based on a seed layer. c) AFM images of the hydrogel formed from the surface at 120 min and 12 h. Reproduced with permission.[35] Copyright 2015, Wiley.

Based on work performed in solution, the same group has established in 2017 that α-chymotrypsin confined on a surface is able to oligomerize ethyl ester dipeptides KL-OEt (K = lysine, OEt = ethyl ester group at C-terminal position) and produce oligopeptides (KL)$_n$-OEt, which are efficient LMWHs at the surface.[36] The growth of the self-assembly network starts after a lag time that can easily be tuned by varying the surface density of α-chymotrypsin and the concentration of KL-OEt in solution. The thickness of the

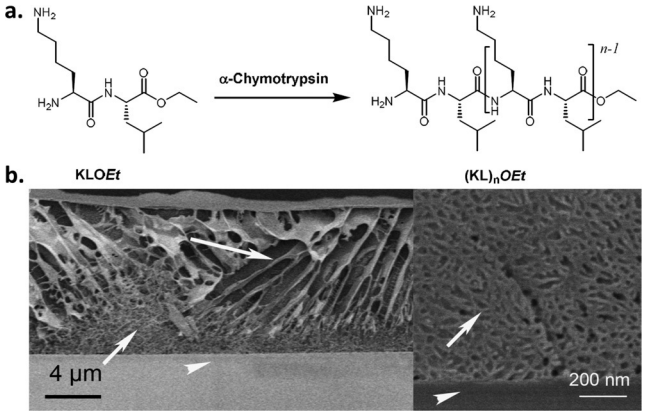

Figure 12. a) Oligomerization of KL-OEt into $(KL)_n$-OEt catalyzed by α-chymotrypsin. b) Cryo-MEB images of the $(KL)_n$-OEt-based hydrogel in *z*-section. Reprinted with permission from Ref. [36]. Copyright 2017 American Chemical Society.

formed fiber network can reach up to circa 10 μm. Cryo-MEB images shows that the gel network is denser at the interface than far from the interface (Figures 12).

4. Applications

In spite of the recent emergence of this field, some applications based on the strategies developed in the previous sections have already been reported. Because of the aqueous nature of the material formed at the interface, a hydrogel, all of these applications are related to the biological field.

Surface-assisted self-assembly relying on the electrodeposition of the pH-responsive hydrogelator was used to prepare an efficient biosensor able to detect the presence of a model substrate, S-adenosyl-homocysteine (SAH), in the surrounding solution.[37] The fabrication of this sensitive matrix was achieved in two steps. First, self-assembly of Fmoc-F-OH was triggered through the application of an anodic current, which generated a localized proton gradient. This process was realized with a warm solution containing the hydrogelator, gelatin, and cells (*E. coli*), leading to their entrapment in the self-assembled structure. When the system was cooled down, an interpenetrated network formed because of the assembly of gelatin chains. In this hybrid network *E. coli* remained alive. Then, the peptide-based scaffold was dissolved by the addition of a basic buffer (pH 8), resulting in a gelatin matrix with embedded cells. By using transglutaminase (mTG), the covalent immobilization of two conjugated enzymes (Pfs and Luxs) was done as well as the cross-linking of the gelatin architecture. In this way, SAH could be transformed through a cascade of enzymatic reactions (catalyzed by enzymes Pfs and Luxs), leading to the signaling molecule AI-2. This compound was recognized by *E. coli*, engineered to express a fluorescent protein (DsRed) upon exposure to AI-2.

More recently, Xu et al. used the surface-induced hydrogelation property of a modified substrate to detect enzyme activities in complex biological fluids.[38] The surface sensor was a glass substrate rendered fully cationic to adsorb hydrogelators. In presence of the anionic peptide NBD-FFY (NBD = *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)) in solution, this peptide self-assembled exclusively on the surface leading to a hydrogel film with intrinsic fluorescence thanks to the π–π stacking of NBD moieties. When the tyrosine residue of this LMWH was phosphorylated, NBD-FFpY did not self-assemble and remained soluble despite the presence of the positively charged substrate. Dephosphorylation of this peptide was done by using phosphatase in solution; the higher the concentration of phosphatase in solution, the thicker the hydrogel layer formed and therefore the more intense the fluoresence measured on the surface. It appears that the fluorescence intensity measured from the formed gel varies linearly with the concentration of enzyme in solution. Because cancer cells such as ovarin cancer lines contain phosphatases, this property was suggested for cancer cell detection.

The most promising development that appeared in the last few years is the localized enzyme-assisted self-assembly around or even inside cells. Based on the presence of endogenous enzymes (mainly alkaline phosphatase) distributed along the plasma membrane of specific cells,[39] it has been demonstrated that in the presence of suitable LMWH, a self-assembled network forms around the cells, which prevents their aggregation and can lead to cell death. This is anticipated to be useful for many applications in biomedicine; for example, for inhibition of bacterial[40] and tumor growth,[41] control of cancer cell fate,[42] and prevention of platelet aggregation by thrombin and other agonists.[43] LMWH can also penetrate and self-aggregate in cells. This is of interest for intracellular imaging and even intratumoral chemotherapy.[44]

5. Summary and Outlook

The surface-assisted self-assembly of LMWH is an emerging field in surface science based on the localized organization of matter leading to supramolecular hydrogel growth. Up to now, two main strategies have been reported to direct the self-assembly from the surface. One can create a seed layer by adsorption or covalent coupling of the hydrogelator molecules onto a substrate. It is now established that such a surface favors the gel formation by self-assembly of the LMWH. However, the molecular organization that initiates gelation at the interface at concentrations significantly lower than the CGC of gelation in the bulk is still poorly understood. All the publications reporting this effect mention only a local increase of the hydrogelator concentration initiated by the surface without discussing the molecular mechanism leading to the gel formation. In particular, for a given hydrogelator system, what is the relationship between the surface density of adsorbed or anchored hydrogelator molecules and the critical concentration at which gelation occurs at a surface? Another issue is the relationship between the strength of the interactions between hydrogelator molecules and this critical concentration. What is the role of lateral interactions between self-assembled fibers in the control of the localized hydrogel formation? It

would also be of great interest to use molecular simulations that could give information on the molecular mechanism that gives rise to the self-assembly process.

The second way to initiate localized self-assembly leading to gel formation is by generating the hydrogelator molecules locally at the surface. This was done either by electrochemistry or by surface-localized catalysis.

Electrochemically induced self-assembly has great potential in the design of biosensors because it allows easily addressing different microelectrodes independently on a single chip. However, up to now, all studies mainly focused on proofs of concept rather than fundamental issues. A few fundamental issues must be addressed before this technique can be used to routinely functionalize electrodes. A precise modeling of how the pH gradients develop near the surface as a function of various parameters, such as current density, application time, and presence of a localized gel, is missing. The self-assembled gels are out-of-equilibrium systems because after rinsing with water or buffer solutions they remain stable. This implies that the history of how they were formed can be of importance to their structure. In particular, because they are generated from a pH gradient, do they show a fiber density gradient or a gradient in the size of the fibers? What is the relationship between network structure and current density? Finally, all studies reported so far were based on the production of protons to induce gelation. Other systems besides those based on pH gradients should be developed that allow for an electrochemical buildup of a localized LMWH hydrogel.

Localized self-assembly through surface catalytic reactions, in contrast to the electrochemical approach, can be applied on almost any kind of substrate and any geometry, in particular on micro- and nanoparticles. Because the material generated from the self-assembly is a hydrogel, many exciting perspectives of this field can be foreseen in biomedical sciences and engineering, specifically in the design of new functional biomaterial coatings. The network structure of the LMWH assemblies is particularly appealing for biological applications since it mimics the fibrous nature of the extracellular matrix.[45] However, many fundamental issues need to be addressed before the behavior of a system can be predicted. First, one has to be able to well characterize surfaces covered by enzymes (surface concentration, enzyme distribution on the surface, and enzymatic activity of the surface). All these parameters are anticipated to play a critical role in the self-assembly process. One has also to understand how the self-assembled structures interact with the surface. First studies showed that for enzymes deposited on the surface in the form of isolated aggregates, fibers emerged from the aggregates in the form star-like structures.[32] This implies a strong interaction between the hydrogel molecules and the enzyme aggregates. What happens when the enzymes are distributed continuously on the surface?

Localized self-assembly processes play an important role in positioning the different organelles in cells,[46] in localizing the cellular chemical reactions,[47] and in adapting the cell to its environment.[48] These processes take place in highly crowded media. Up to now, localized self-assembly processes were only developed in aqueous solutions. It would be of particular interest to develop them in host hydrogels, on the surface of particles or vesicles. This is possible using enzyme-assisted localized self-assembly. It could open the route towards the design of highly structured 3D hydrogels and would constitute a first step toward the buildup of materials that mimic morphogenetic tissue growth. Such self-assembled gels would also be essential bricks for the construction of artificial cells.

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Conflict of interest

The authors declare no conflict of interest.

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